WHEN PHENOLIC ACIDS BECOME PRO-OXIDANT DURING *IN VITRO* DIGESTION OF A LOW-FAT AND HIGH-FAT BEEF PRODUCT

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Abstract – In this study, we tested the effect of 4 phenolic acids (caffeic, α-ferulic, chlorogenic and gallic acid) on the lipid oxidation parameters malondialdehyde (MDA), 4-hydroxy-nonenal (4-HNE) and hexanal (HEX) during *in vitro* digestion of a low-fat and high-fat beef product. Higher concentrations of malondialdehyde (10-fold), 4-hydroxy-nonenal (14-fold) and hexanal (8-fold) were found in the high-fat beef digests compared to the low-fat beef digests. During digestion of the low-fat beef product, all phenolic acids decreased the formation of lipid oxidation products. Addition of phenolic acids during the high-fat beef digestion resulted in either pro-oxidant or antioxidant effects, depending on their concentration. This study suggests that consumption of high-fat meat products is associated with a higher health risk than low-fat meat products, and that the simultaneous consumption of phenolic acids may have a dual role.

Key Words – Meat - Lipid oxidation – Health – Digestion

I. INTRODUCTION

High consumption of processed meats has been reported to increase the risk of several oxidative stress-related diseases such as cancer, diabetes and cardiovascular diseases. Previously, we demonstrated the increased formation of the lipid oxidation products (LOP) malondialdehyde (MDA), 4-hydroxy-2-nonenal (4-HNE) and hexanal (HEX) during *in vitro* digestion, when processed meat contained a high haem-Fe content, a high fat content or was heated [1,2,3]. Malondialdehyde has been shown to be absorbed following consumption of turkey cutlets [4], where after it could reach sensitive tissues and cause damage to cells and DNA.

Strategies to inhibit these oxidation processes during the digestion of processed meat may have health benefits. In this study, we aimed at elucidating whether phenolic acids could alleviate oxidative reactions throughout the digestion of a lean and fat cooked beef product. For this purpose, we tested the addition of different concentrations of phenolic acids during *in vitro* digestion of a lean and fat beef product. Sirota *et al.* [5] showed that the formation of MDA during *in vitro* digestions was highly predictive for the *in vivo* effect of polyphenols on postprandial plasma MDA in humans.

II. MATERIALS AND METHODS

Lean meat samples from the *m. Pectoralis profundus* of beef were manually chopped into cubes of approximately 1-2 cm^3, and used for the digestion of the low-fat beef product (1% fat). A high-fat beef product was obtained by adding lard to the muscle up to an estimated total fat content of 15%. Meat products were minced in a grinder (Omega T-12) equipped with a 10 mm plate, followed by grinding through a 3.5 mm plate and heated in a warm water bath for 15 min after the core temperature had reached 65°C. After manufacturing, all meat samples were homogenized in three 5s bursts using a food processor (Moulinex DP700), vacuum packed and stored at -20°C until the start of the incubation.

The digestive simulations were performed, based on our previously described *in vitro* digestion protocol, specific for studying oxidation processes during passage through the gastrointestinal tract [1]. Digestions consisted of an enzymatic digestion simulating mouth, stomach and duodenal gastro-intestinal tract compartments, whereby 4.5

g of the low-fat or high-fat beef product was incubated with 0, 2.5, 5, 10 or 20 mg of each of the phenolic acids.

MDA concentrations in digesta were measured spectrophotometrically by a modified method in accordance with Grotto *et al.* [6]. Levels of 4-HNE and HEX were analyzed in digesta after formation of their fluorometric derivatives with 1,3-cyclohexanedione through HPLC (Agilent 1200 series, equipped with a degasser, auto sampler, quaternary pump, column oven, fluorescence detector) as previously described by Van Hecke *et al.* [1]. Al data are expressed relatively to control.

Data on MDA, 4-HNE and HEX were analyzed for each phenolic compound and meat type separately, using a one-way ANOVA with the fixed factor of compound concentration. Post hoc comparisons were performed using the Tukey HSD test. P-values lower than 0.05 were considered to be statistically significant.

III. RESULTS AND DISCUSSION

A clear difference in all LOP ($P<0.001$) was observed between the digested low-fat and high-fat beef product in the mimicked duodenum phase (Figure 1), confirming our previously reported results [2].

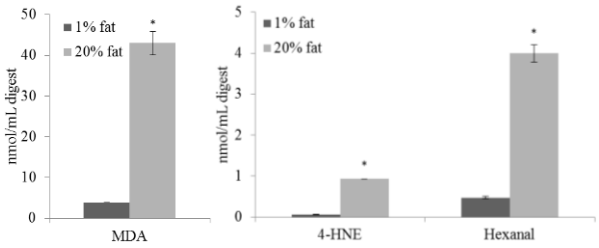

Figure 1: Digestion of the high-fat beef product resulted in 10-fold higher MDA, 14-fold higher 4-HNE and 8-fold higher HEX concentrations, compared to the low-fat beef product.

During the digestion of the low-fat beef product, all phenolic acids demonstrated an antioxidant effect, plausibly by scavenging ROS produced during the Fenton reaction. However, caffeic acid showed to be less efficient when added at higher doses (Figure 2).

During the digestion of the high-fat beef product, all phenolic acids decreased MDA values ($P<0.001$), albeit to a lesser extent for chlorogenic acid (Figure 3). On the other hand, a peak in 4-HNE formation was observed with a significant increase between 35-53% when α-ferulic- and chlorogenic acid were applied at the lowest dose, after which it gradually decreased with increasing doses. A trend for a higher 4-HNE formation at the lowest dose of caffeic acid was observed ($P=0.066$). Only doses of 10 and 20 mg caffeic acid and 20 mg α-ferulic acid decreased 4-HNE respectively down to 21 and 58% of the control.

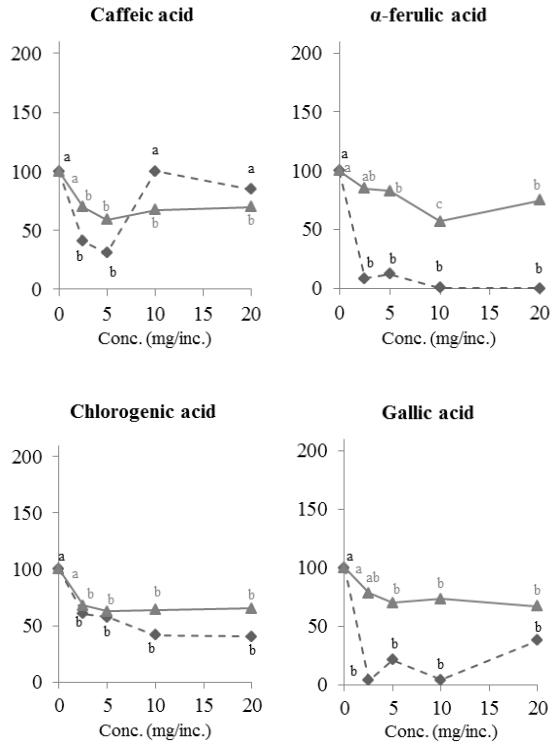

Figure 2: Effect of different doses (0, 2.5, 5, 10, 20 mg/incubation) of caffeic, α-ferulic, chlorogenic and gallic acid on malondialdehyde (◆) and hexanal (▲) proportions (% relatively to control) in duodenal digested beef with 1% fat. Data labels refer to significant differences ($P<0.05$) among concentrations.

In contrast to the other phenolic acids, there was a dose dependent increase of 4-HNE with increasing dose of gallic acid during the digestion of the high-fat beef product.

Addition of doses up to 10 mg chlorogenic acid to the high-fat beef caused an increase in HEX concentrations up to 30% after which a decrease to the level of the control was observed at the highest dose ($P=0.019$). The other phenolic acids caffeic acid and α-ferulic acid demonstrated a dose-

dependent HEX decrease down to 17 and 43% (resp. P<0.001 and P=0.004) of the control, whereas gallic acid had no effect on HEX formation.

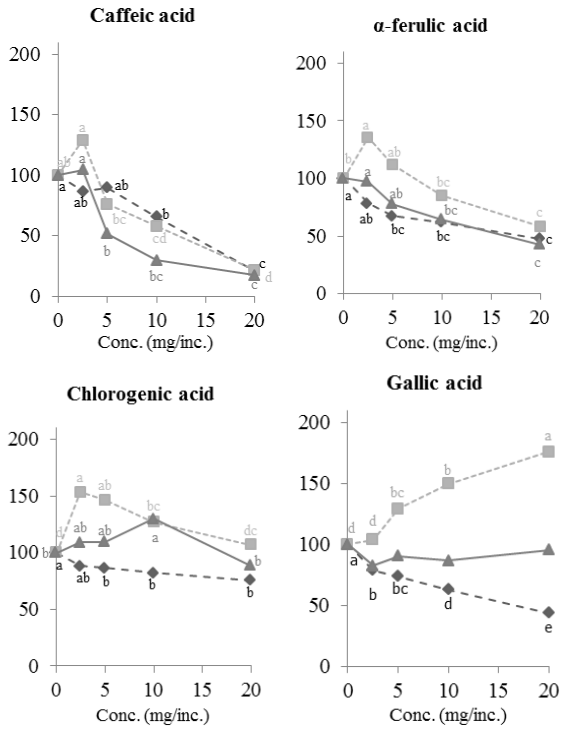

Figure 3: Effect of different doses (0, 2.5, 5, 10, 20 mg/incubation) of caffeic, α-ferulic, chlorogenic and gallic acid, on malondialdehyde (◆), 4-hydroxy-nonenal (■) and hexanal (▲) proportions (% relatively to control) in duodenal digested beef with 15% fat. Data labels refer to significant differences (P<0.05) among concentrations.

The effect of the addition of phenolic acids during *in vitro* digestion on LOP formation thus clearly depended on the matrix in which it was added. Previously, we showed that water soluble reducing agents were very effective antioxidants during the digestion of low-fat beef, but demonstrated pro-oxidant effects during high-fat beef digestion [7]. On the other hand, fat soluble reducing agents such as α-tocopherol, silibinin and quercetin showed to be effective antioxidants during high-fat beef digestion.

The Fenton reaction, which initiates oxidation, is catalyzed by the highly hydrophilic H_2O_2 [8] and therefore, will mainly occur in the aqueous phase of the reaction medium. Hydrophilic reducing agents that are able to reduce Fe^{3+} to Fe^{2+} will therefore stimulate the Fenton reaction and generate more ROS. During the digestion of the low-fat beef, the produced ROS can simultaneously be scavenged by the added hydrophilic antioxidants. However, when a large lipid fraction is present, like in the high-fat beef product, ROS that are generated in the aqueous compartment may migrate into the lipid compartment, where they cannot be reached by the hydrophilic antioxidants and hence stimulate the oxidation of n-3 and n-6 PUFAs. On the other hand, lipophilic compounds are not able to stimulate the Fenton reaction in the aqueous phase and can easily scavenge ROS that enter the lipid compartment and hence prevent lipid oxidation [3].

The phenolic acids caffeic acid and α-ferulic acid were described to be nearly 100% hydrophilic in both water/oil systems and emulsions at neutral pH but their partitioning in the water compartment decreased down to 64% when the pH of the system was dropped to 3 [9], similar to the stomach pH during digestion. This partitioning effect was not observed for gallic acid, which remained nearly 100% in the water fraction in all systems. No data was found in the literature on the partitioning of chlorogenic acid in acid heterophasic systems, but a similar partitioning behavior as caffeic acid and α-ferulic acid may be expected.

During the acid stomach digestion, caffeic acid and α-ferulic acid partition in both the water and the lipid phase, thereby stimulating ROS formation from the water compartment by reducing Fe^{3+} to Fe^{2+} [10] and inhibiting lipid oxidation from the lipid compartment. Higher doses of caffeic acid and α-ferulic acid may decrease lipid oxidation compared to the lowest dose since higher concentrations would partition in the lipid compartment and higher concentrations in the water phase could not only reduce Fe^{3+} to Fe^{2+} but also scavenge produced ROS. Indeed, an increase in 4-HNE was observed when caffeic acid, α-ferulic acid and chlorogenic acid were added at the lowest dose, after which a gradual decrease occurred when higher doses were applied. This LOP peak formation at a low dose was not found during the digestion of the low-fat beef product. It is however not clear why this peak formation was not observed for HEX (except for chlorogenic acid) and for MDA during the high-fat beef digestion.

A confounding factor in explaining the mechanisms involved, is the degree of oxidation in the meat samples before digestion. The cooked high-fat beef product was initially already more oxidized than the low-fat beef product. Yamanaka *et al.* [11] observed an antioxidant effect when adding caffeic acid to the initiation phase of Cu^{2+}-induced LDL oxidation. When added during the propagation phase, caffeic acid exerted pro-oxidant activity at lower doses and antioxidant activity at higher doses, similar to our results. Since heating of meat is responsible for increased oxidative reactions during meat processing [3], it might be possible that adding water soluble antioxidants before heating results in a different outcome than the effects reported in this study.

IV. CONCLUSION

In conclusion, our results suggest that phenolic acids can exhibit antioxidant as well as pro-oxidant effects depending on their concentration and the matrix to which they are added. Oxidation processes during digestion are determined by a complex interplay between the "polar paradox theory", the extent of initial oxidation and composition of the meat, and the concentration and nature of the added antioxidants. Studying the partitioning behavior of antioxidants in acid heterophasic systems, such as present in the stomach, would improve our insight on their mode of action.

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REFERENCES

1. Van Hecke, T., Vanden Bussche, J., Vanhaecke, L., Vossen, E., Van Camp, J., & De Smet, S. (2014a). Nitrite Curing of Chicken, Pork, and Beef Inhibits Oxidation but Does Not Affect N-Nitroso Compound (NOC)-Specific DNA Adduct Formation during *in Vitro* Digestion. Journal of Agricultural and Food Chemistry, 62(8), 1980-1988.
2. Van Hecke, T., Vossen, E., Vanden Bussche, J., Raes, K., Vanhaecke, L., & De Smet, S. (2014b). Fat Content and Nitrite-Curing Influence the Formation of Oxidation Products and NOC-Specific DNA Adducts during *In Vitro* Digestion of Meat. *PLOS ONE*, 9(6), e101122.
3. Van Hecke, T., Vossen, E., Hemeryck, L.Y., Vanden Bussche, J., Vanhaecke, L., De Smet, S. (2015). Increased Oxidative and Nitrosative Reactions During Digestion Could Contribute to the Association between Well-done Red Meat Consumption and Colorectal Cancer. *Food Chemistry, DOI: 10.1016/j.foodchem.2015.04.029*
4. Gorelik, S., Ligumsky, M., Kohen, R., & Kanner, J. (2008). The stomach as a "bioreactor": when red meat meets red wine. *Journal of Agricultural and Food Chemistry*, 56(13), 5002-5007.
5. Sirota, R., Gorelik, S., Harris, R., Kohen, R., & Kanner, J. (2013). Coffee polyphenols protect human plasma from postprandial carbonyl modifications. *Molecular Nutrition & Food Research*, 57(5), 916-919.
6. Grotto, D., Santa Maria, L. D., Boeira, S., Valentini, J., Charão, M. F., Moro, A. M., Nascimento, P.C., Pomblum, V.J & Garcia, S. C. (2007). Rapid quantification of malondialdehyde in plasma by high performance liquid chromatography–visible detection. *Journal of Pharmaceutical and Biomedical Analysis*: 43 (2), 619-624
7. Van Hecke, T., Vossen, E., Claeys, E., De Smet, S. (2014c). The presence of a fat compartment during the digestion of beef alters ascorbic acid from antioxidant to pro-oxidant behavior. Les 15èmes Journées Sciences du Muscle et Technologies des Viandes (JSMTV), 4-5 November 2014, Clermont-Ferrand, France.
8. Cordeiro, R. M. (2014). Reactive oxygen species at phospholipid bilayers: Distribution, mobility and permeation. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, 1838(1), 438-444.
9. Schwarz, K., Frankel, E. N., & German, J. B. (1996). Partition behaviour of antioxidative phenolic compounds in heterophasic systems. *Lipid/Fett*, 98(3), 115-121.
10. Moran, J. F., Klucas, R. V., Grayer, R. J., Abian, J., & Becana, M. (1997). Complexes of iron with phenolic compounds from soybean nodules and other legume tissues: prooxidant and antioxidant properties. *Free Radical Biology and Medicine*, 22(5), 861-870.
11. Yamanaka, N., Oda, O., & Nagao, S. (1997). Prooxidant activity of caffeic acid, dietary non-flavonoid phenolic acid, on Cu^{2+}-induced low density lipoprotein oxidation. FEBS letters, 405(2), 186-190.